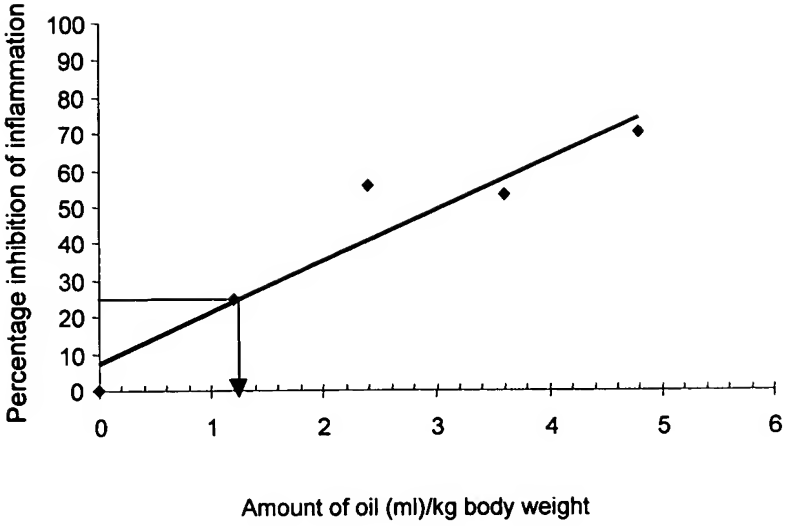


Fig 1. Results from Fig 6 (following) have been re-graphed to establish a relationship from which the inhibitory dose (ID_{25}) of an emu oil can be derived. It remains to be noted that this assay is based on one injection of emu oil. It therefore represents an underestimation of the activity when applications for several days are being used.

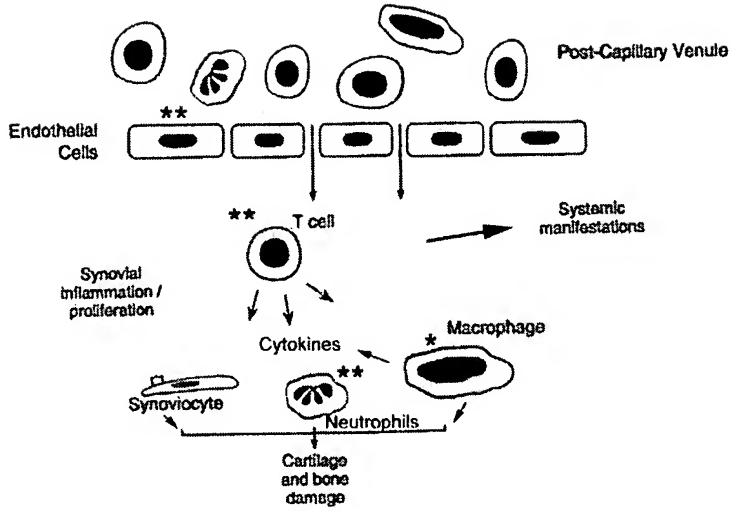
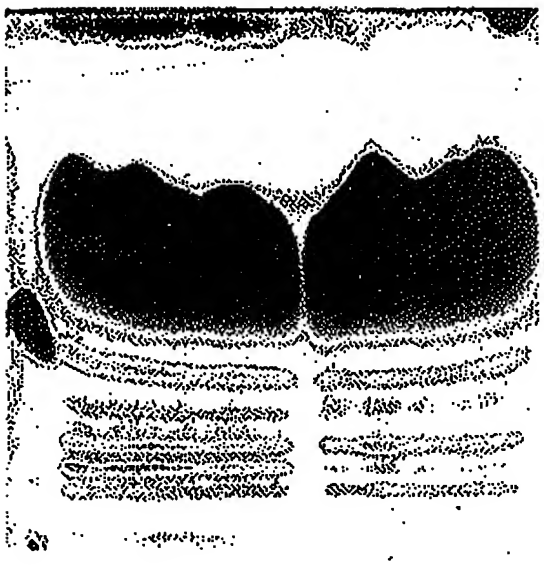


*Fig 2. A schematic representation of the cellular inflammation involved in joint cartilage damage in rheumatoid arthritis. During the disease the endothelium becomes activated and promotes the adhesion and movement of the leukocytes, T cells, neutrophils and monocytes (macrophages) into the joint. A network of intercellular signalling involving cytokines and other mediators, causes the changes of these inflammatory cells to a tissue destructive phenotype. Points of target by emu oil are shown by asterix. ** indicates strong inhibition and * modest inhibition.*



Olive Oil *Emu Oil*

Fig 3. The preparative separation of emu oil by thin layer chromatography (TLC). The oil preparation was loaded onto a silicone-coated TLC plate. The TLC was run in a solvent system consisting of diethylether:hexane:glacial acetic acid (20:80:1) until 1 cm from the margin. The plate was dried and samples covered with foil for protection and the zones developed by exposure to I_2 gas.

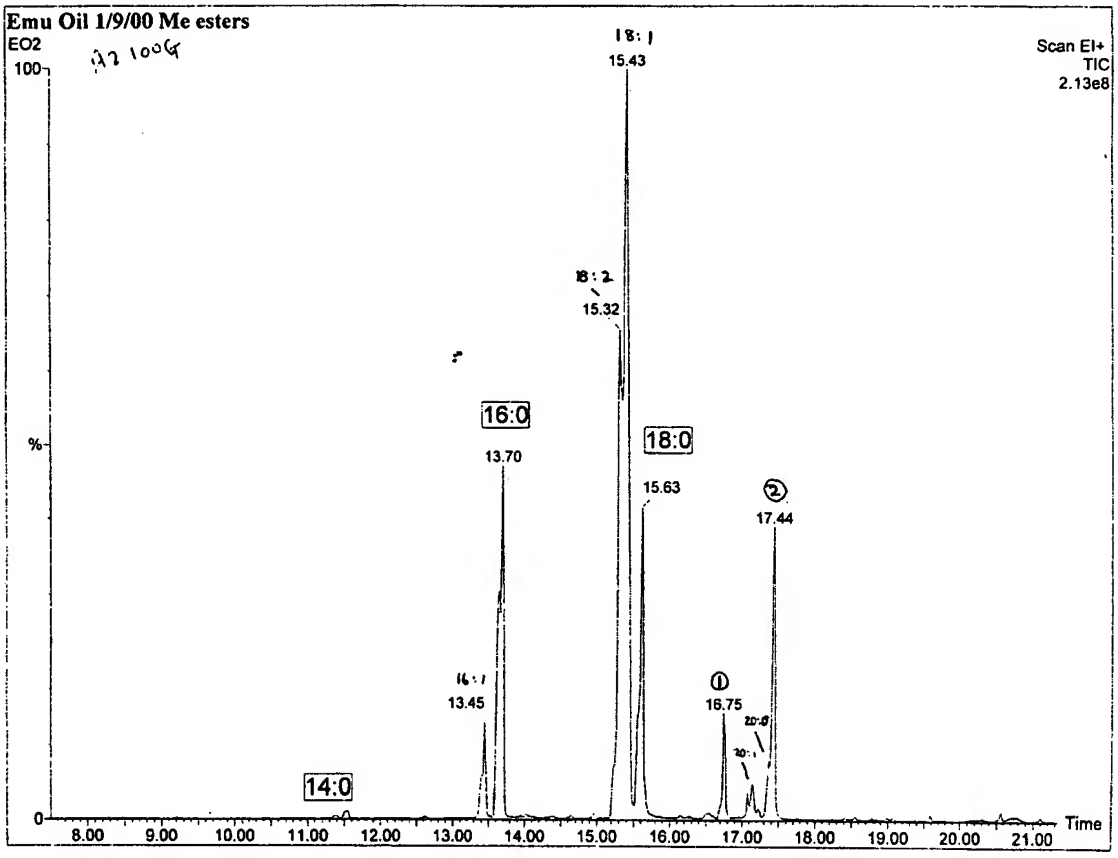


Fig 4. GC-MS analysis of A2-100G emu oil. Trans-esterification of emu oil components was carried out at 90℃ for 90 min in benzene/acetylchloride/methanol.

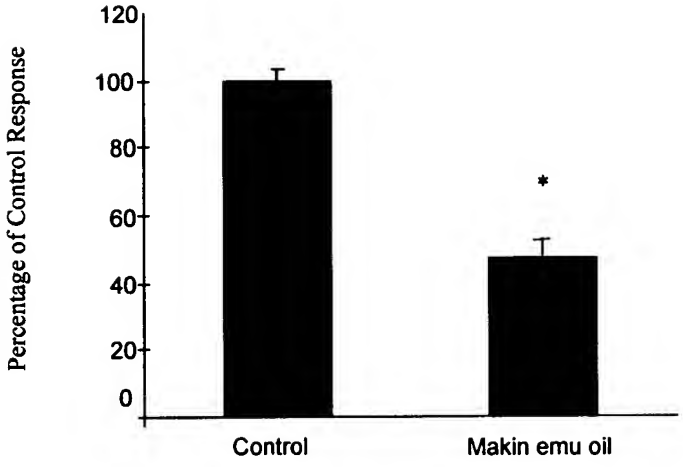


*Fig 5. The effect of Makin emu oil on the DTH response. Mice were immunised with SRBC subcutaneously and then challenged with SRBC in the footpad after 5 days. Three hours prior to the challenge, the mice received 50μl of Makin emu oil intraperitoneally (ip). The reaction (footpad swelling) was measured 24 h after challenge by dial calliper. Controls received 50μl of saline. The data represent Mean ± sem of 5 mice. The Mean ± sem increase in footpad swelling of control was 0.75 ± 0.02mm. Statistical analysis by Student's t-test, * P < 0.0001 for comparison between Control and Makin emu oil.*

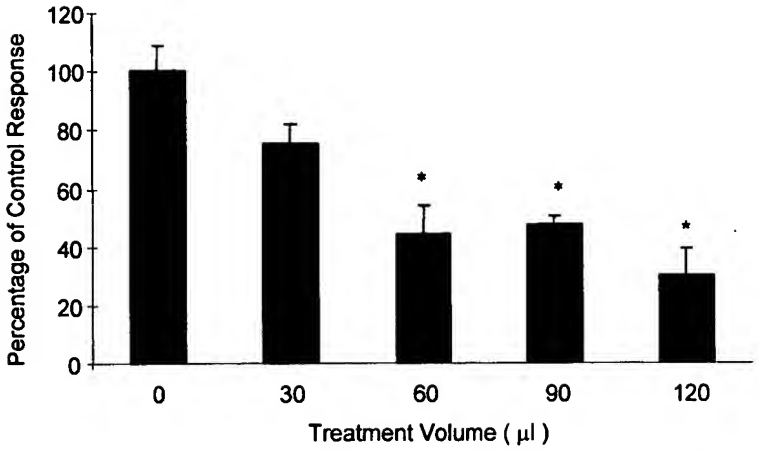


*Fig 6. The effects of varying amounts of emu oil on the DTH response. Mice were immunized with SRBC subcutaneously and then challenged with SRBC in the footpad on day 5. Three hours prior to the challenge, the mice received various doses of Makin emu oil ip. The reaction was measured 24h after challenge by dial calliper. The data represent Mean ± sem of 5 mice. The Mean ± increase in the footpad swelling of control was 1.17 ± 0.10mm. Statistical analysis by Dunnett's test for multiple comparisons, * P < 0.01 for comparison between Control and tests.*

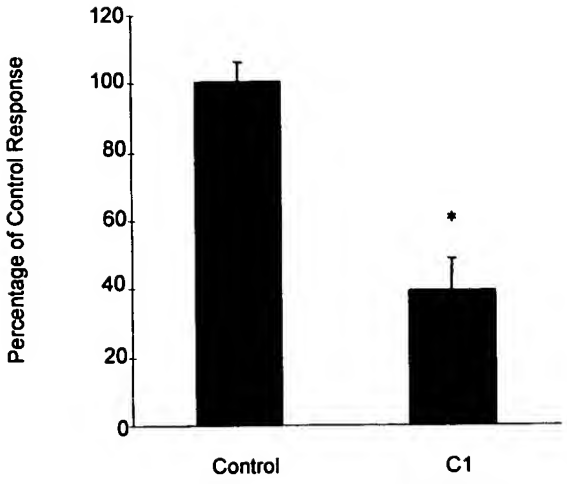


*Fig 7. The effect of a commercial emu oil cream (C1) on the DTH response. Mice were sensitised subcutaneously with SRBC and challenged 5 days later in the footpad. 1 hour prior to challenge, the mice received C1 emu oil applied topically to the footpads and tail. The footpad thickness was measured 3h after challenge by dial calliper. The results are the Mean ± sem of 5 mice. The Mean ± sem swelling of controls was 0.7 ± 0.04 mm. Statistical analysis by Student's t-test, * P = 0.0005 for comparison between Control and commercial emu oil cream.*

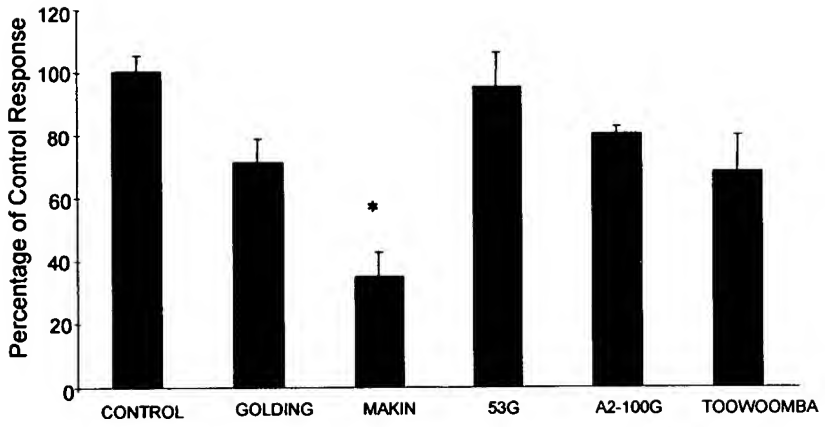


*Fig 8. The effect of various emu oil preparations on the DTH response. Mice were immunised with SRBC subcutaneously and then challenged on day 5 in the footpad. Three hours prior to challenge, the mice received 100µl of emu oil ip. The reaction was measured 24h after challenge by dial calliper. The data represent Mean ± sem of 5 mice. The Mean ± sem of control was 1.05 ± 0.01 mm. Statistical analysis by Dunnett's test, *P < 0.01 for comparison between Control and Makin emu oil.*

***NB** GOLDING = LITTLE MEADOW*

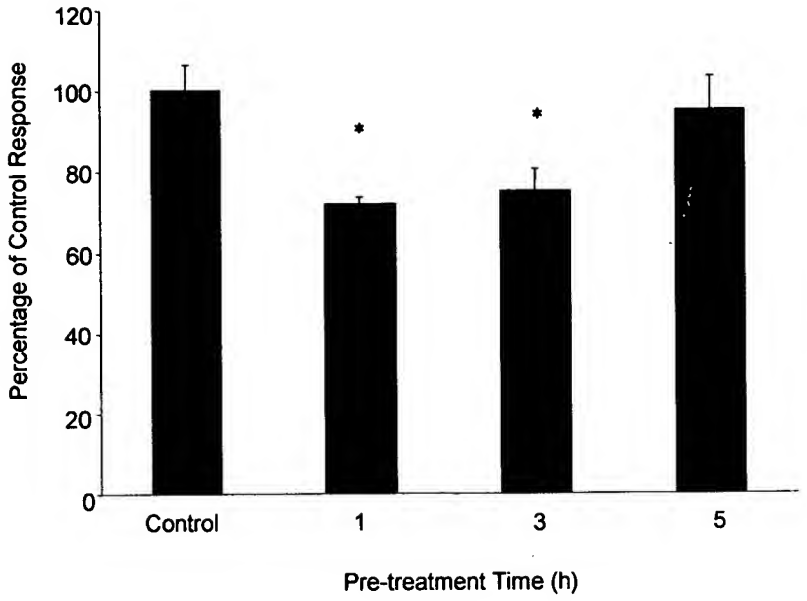


*Fig 9. Effect of Makin emu oil pretreatment time on the DTH response. Mice were immunised with SRBC subcutaneously and then challenged with SRBC on day 6. Mice were treated ip with 50μl of Makin emu oil at 1, 3 or 5 h prior to challenge. The swelling of the footpad was measured 24h after challenge by dial calliper. Controls received 50μl of PBS. The data represent Mean ± sem of 5 mice. The Mean ± increase in footpad swelling of control was 1.03 ± 0.07 mm. Statistical analysis by Dunnett's test for multiple comparisons, * $P < 0.05$ for comparison between Control / 1h and Control / 3h pre-treatment times.*

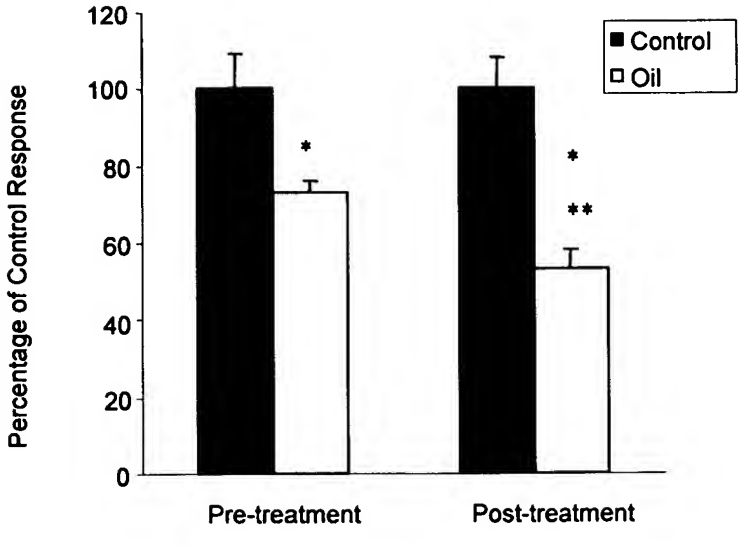


*Fig 10. The effect of Makin emu oil pre-treatment and post-treatment on the DTH response. Mice were injected ip with 120µl of Makin emu oil either 4h before (pre-treatment) or 3h after (post-treatment) challenge. Control mice received PBS. The swelling of the paw was measured after 24h by dial calliper. The results are the Mean ± sem of 5 mice. The Mean ± sem of controls was 1.18 ± 0.09mm. Statistical analysis by Student's t-test, * P = 0.01 for comparison between Makin pre - treatment and Control and post-treatment and Control; * P = 0.01 for comparison between Makin pre-treatment / post-treatment; ** P < 0.01 for comparison between Control and Makin post-treatment.*

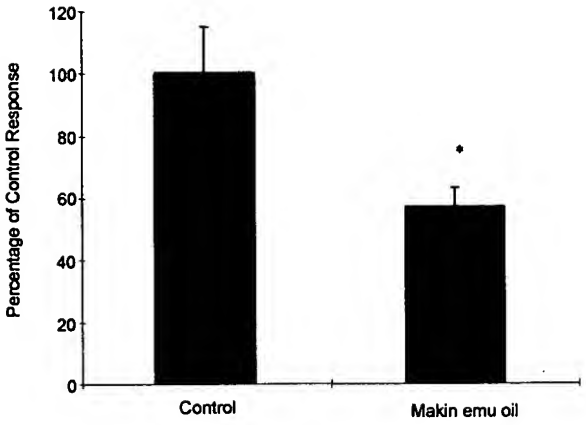


*Fig 11. The effect of Makin emu oil on the Carrageenan-induced paw inflammation. Mice were injected ip with 50µl of Makin emu oil. The animals were injected 3h later in the hind footpad with 25µl of 1% Carrageenan. Control mice received PBS instead of oil. The swelling of the paw was measured after 24h by dial calliper. The results are the Mean ± sem of 5 mice. The Mean ± sem of controls was 0.74 ± 0.11mm. Statistical analysis by Student's t-test, * P = 0.001 for comparison between Control and Makin emu oil.*

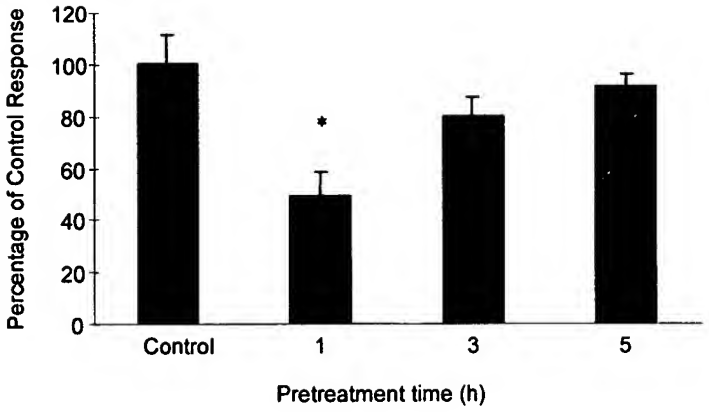


*Fig 12. The effect of Makin emu oil pretreatment time on Carrageenan-induced paw oedema. Mice were injected ip with 50μl of either Makin emu oil or PBS 1h, 3h, or 5h before injecting the hind footpad with 25μl of a 1% solution of Carrageenan in PBS. The swelling of the paw was measured after 3h by dial calliper. The results are the Mean ± sem of 5 mice. The Mean ± sem of controls was 0.7 ± 0.07 mm. Statistical analysis by Dunnett's test for multiple comparisons, * $P < 0.01$ for comparison between Control and 1h pre-treatment time with Makin emu oil.*

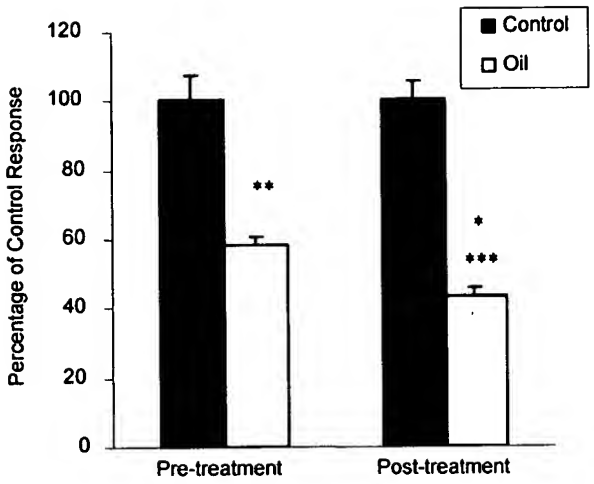


*Fig 13. The effect of Makin emu oil pretreatment and post-treatment on the Carrageenan-induced paw inflammation. Mice were injected ip with 120µl of Makin emu oil 3h before or 3h after challenge by injection of 1% Carrageenan into the hind footpad. Control mice received PBS. The swelling of the paw was measured after 24h by dial calliper. The results are the Mean ± sem of 5 mice. The Mean ± sem of controls were 0.9 ± 0.17mm and 0.76 ± 0.13mm respectively. Statistical analysis by Student's t-test, * P = 0.04 for comparison between Makin emu oil pre/post-treatment; ** P =0.002 for comparison between Control and Makin emu oil pre-treatment; *** P =0.001 for comparison between Control and Makin emu oil post-treatment.*

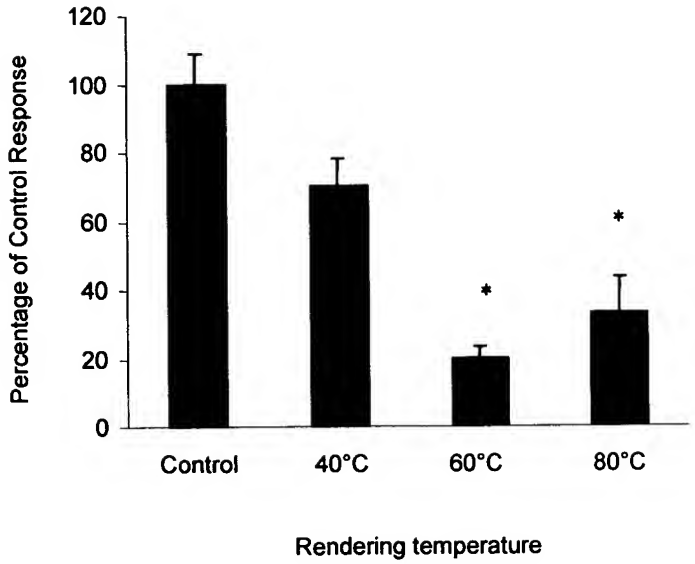


*Fig 14. The effect of temperature of rendering of emu oil on the Carrageenan-induced DTH response. Groups of mice were treated with 120μl of oil prepared in the laboratory by rendering fresh emu fat at 40°C, 60°C or 80°C respectively, 3h prior to injection with 25μl of 1% Carrageenan in PBS into the hind footpad. Control mice received PBS. The swelling of the footpad was measured after 24h. The results are the Mean ± sem of 4 mice. The Mean ± sem of controls was 0.83 ± 0.07mm. Statistical analysis by Dunnett's test for multiple comparisons, *P < 0.01 for comparison between Control and Makin emu oil rendered at 60°C and 80°C.*

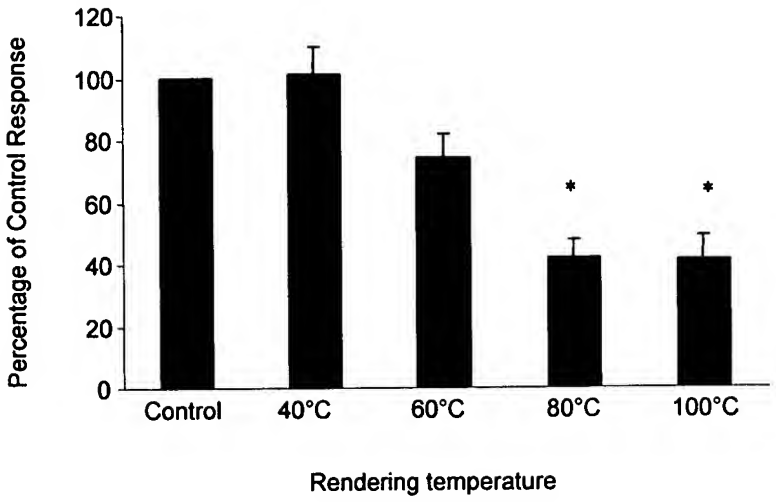


*Fig 15. The effect of rendering temperature of emu oil produced in the laboratory on the DTH response. Oils were prepared on two separate occasions from an emu fat mixture sourced from both gut and back fat locations. Fresh emu fat was heated at 40^{0}C for 2h and the oil collected. The fat remaining was then re- heated at 60^{0}C and further oil collected. The process was repeated at 80^{0}C. Each fraction of oil was tested on the DTH response. Mice were immunised with SRBC subcutaneously and then challenged with SRBC in the footpad after 5 days. Three hours prior to challenge, the mice received ip oil rendered at 40^{0}C, 60^{0}C or 80^{0}C . The reaction was measured 24 h after challenge by dial calliper. Controls received 120µl of PBS. The data represent Mean ± sem of 8 mice. The Mean ± sem increase in footpad swelling of controls was 1.21 ± 0.17 mm. Statistical analysis by Dunnett's test for multiple comparisons, * P < 0.01 for comparison between Control and Makin emu oil rendered at 80^{0}C and 100^{0}C.*

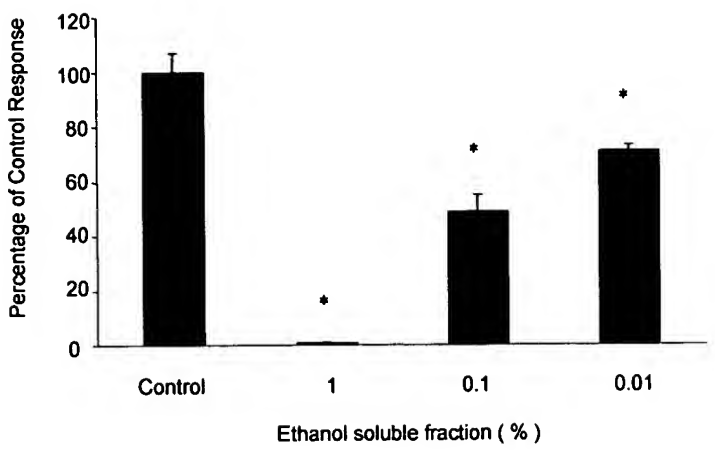


*Fig 16. Effect of ethanol extract of Makin emu oil on lymphoproliferation. To 50µl of purified T lymphocytes (4 x 10^6 /ml) was added an equal volume of ethanol extract of Makin emu oil to the indicated final amount (%) equivalent of whole emu oil. The cells were incubated at 37^{0}C / 5% CO_2 / humid atmosphere for 30min before 100µl of 5% AB serum or 2µg/µl PHA (in 5% AB serum) was added to the wells. The wells were then incubated at 37^{0}C / 5% CO_2/ humid atmosphere for 48 h. Six hours prior to harvesting, cells were pulsed with 1µCi of methyl-^{3}H-thymidine. Incorporated radioactivity was measured using a β counter. The proliferation stimulation index in controls (absence of emu oil extract) was 44. Statistical analysis by Dunnett's test for multiple comparisons, * P < 0.01 for comparison between Control and test samples.*

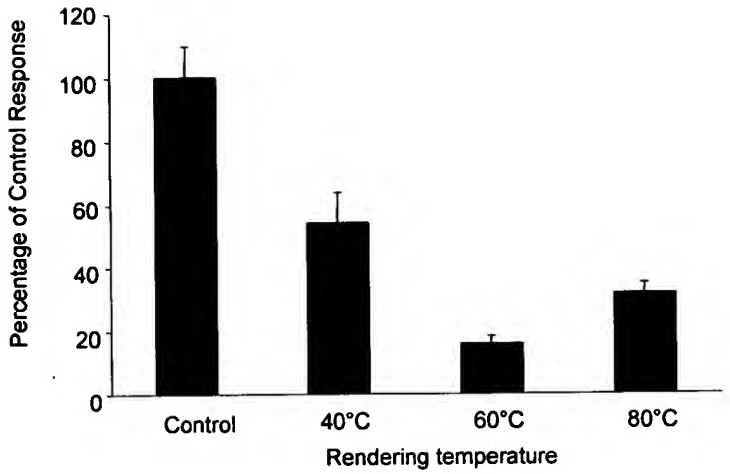


Fig 17. The effects of rendering temperature on the anti-lymphoproliferative effects of emu oil prepared in the laboratory. Oils were prepared from emu back fat. A piece of fresh fat was heated at 40°C for 2h and the oil collected. The remaining fat was then heated at 60°C and further oil collected. The process was repeated at 80°C. Each fraction of oil was subjected to ethanol extraction and then tested for anti-lymphoproliferative activity. To a volume of 50µl of purified T lymphocytes at 4 x 10^6/ml was added an equal volume of the ethanol extract to give 1% final of the whole emu oil equivalent. The cells were incubated at 37°C/5% CO_2/ humid atmosphere for 30min before 100µl of 5% AB serum or 2µg/µl PHA (in 5% AB serum) was added to the wells. The wells were then incubated at 37°C/ 5% CO_2/ humid atmosphere for 48h. Six hours prior to harvesting, cells were pulsed with 1µCi of methyl - ^3H -thymidine. Incorporated radioactivity was measured using a β counter. The proliferation stimulation index in controls (absence of emu oil extract) was 147. Statistical analysis by Dunnett's test for multiple comparisons, $P < 0.01$ for comparison between Control and test samples. Comparison by Tukey-Kramer Test for multiple comparisons, 40°C vs 60°C ($P<0.001$) and 40°C vs 80°C ($P<0.001$).

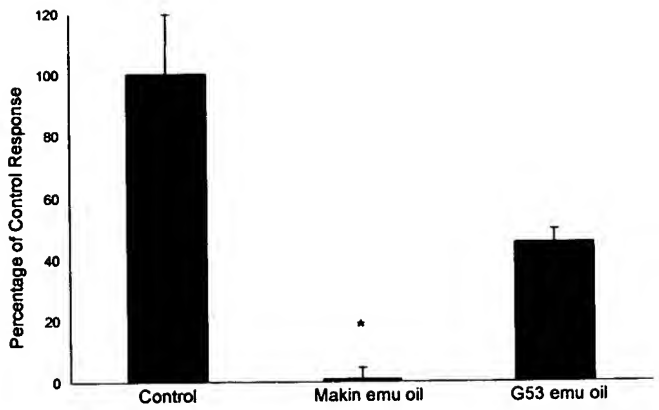


Fig 18. Comparison of the effects of ethanol soluble fractions from Makin and G53 emu oil on lymphoproliferation. Human peripheral blood mononuclear leukocytes (MNL) were treated with the fractions (equivalent to 1% of whole oil) of these two oils for 30 min and then stimulated with the T cell mitogen, phytohaemagglutinin (PHA). The cells were then incubated for a further 48 h and the degree of proliferation assessed by measuring the amount of ^{3}H - thymidine incorporated. The results are mean ± sem of three experiments. The proliferation stimulation index in controls (absence of emu oil extract) was 121. Statistical analysis, $P < 0.01$ by Dunnett's test for multiple comparisons for comparison between Control and Makin emu oil .*

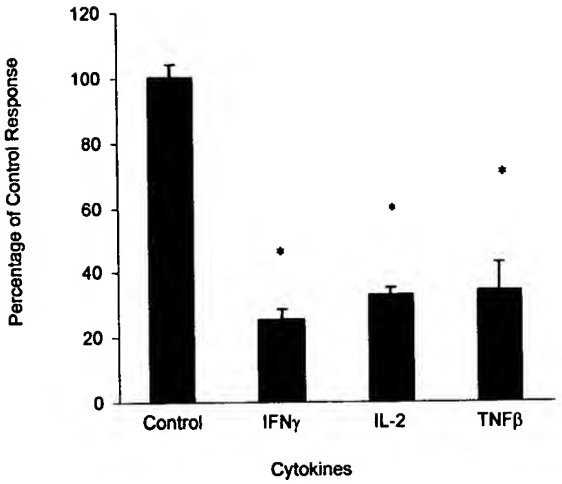


Fig 19. The effect of ethanol extract of Makin emu oil on cytokine production by T lymphocytes. A volume of 50µl of 4 x 10^6/ml of purified T lymphocytes was treated with 50µl of 1% (v/v) of Makin emu oil ethanol extract for 30min, stimulated with 100µl of 2µg/ml PHA in RPMI and incubated at 37^{0}C / 5% CO_2 for 48h. The supernatants were collected and the various cytokines measured by ELISA. The stimulation index in controls (absence of emu oil extract) was 5.6. Statistical analysis by Dunnett's test for multiple comparisons, $P < 0.01$ for comparisons between Control and test samples.*

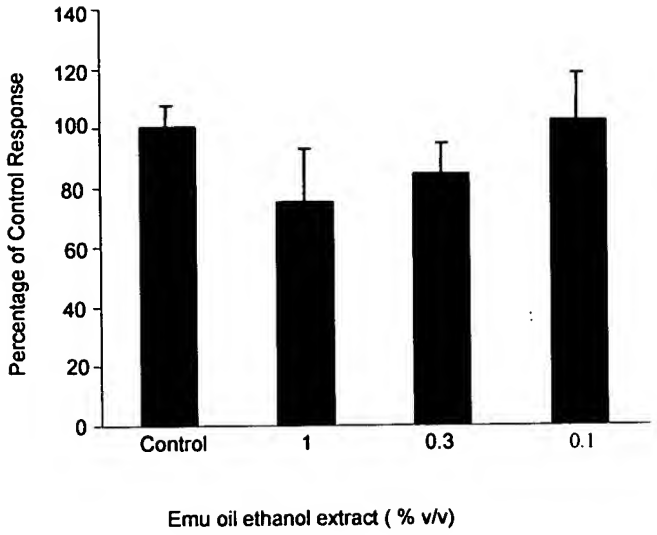


Fig 20. The effect of ethanol extract of Makin emu oil on production of TNF by human monocytes. To 2 x 10^5 peripheral blood mononuclear cells was added 100µl of medium containing the extract to the indicated final amounts (% equivalent of whole emu oil) and the cells incubated for 30min at 37^{0}C in an atmosphere of 5% CO_2. The monocytes were stimulated by the addition of 100µl of 200ng/ml LPS in RPMI and incubated at 37^{0}C / 5% CO_2 for 48h. The supernatant was collected and TNF measured by ELISA. The stimulation index in controls (absence of emu oil extract) was 8.

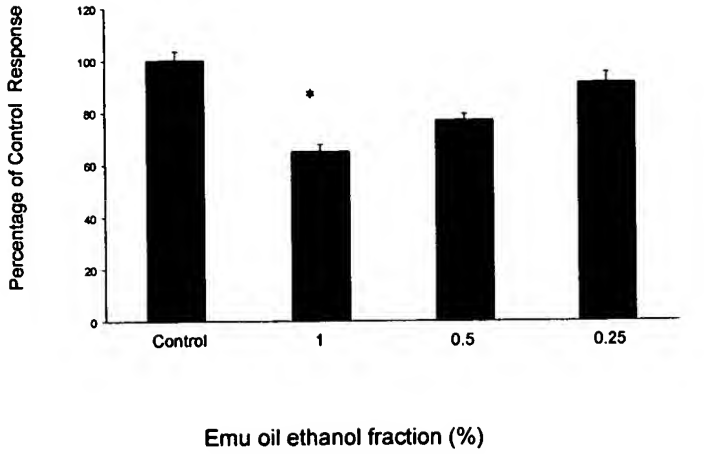


*Fig 21. The effect of Makin emu oil ethanol fraction on adherence of neutrophils to plastic surfaces. An aliquot of 50µl of neutrophils (5 x 10^6 / ml) was treated with 50µl of ethanol fraction at the indicated final amounts (%) equivalent of whole oil for 30min at 37°C/ 5% CO_2 on plates coated with autologous plasma. The cells were stimulated with TNF (125 units / ml) for 30min at 37°C / 5% CO_2 and non-adherent cells were washed three times with HBSS and adherent cells were stained with Rose Bengal. The absorbance was read at 570nm. The stimulation index in controls (absence of emu oil extract) was 3. Statistical analysis by Dunnett's test for multiple comparisons, * $P < 0.01$ for comparison between Control and 1% Makin ethanol extract.*

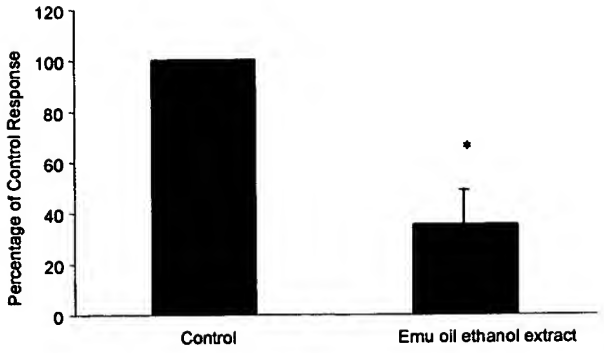


*Fig 22. The effect of Makin emu oil ethanol extract on adhesion of neutrophils to HUVECS. 2 x 10^6 HUVECS in 200µl were seeded in a 96 well plate and cultured for 2 days at 37^{0}C / 5% CO_2. On the day of use the cells were washed three times with 100µl HBSS. 100µl of fresh RPMI containing the ethanol extract (final of 1%, v/v whole oil equivalent) was added. Control wells received ethanol (1% v/v). The cells were incubated for 1h at 37^{0}C / 5% CO_2. Then PMA at final concentration of 10^{-7}M in RPMI with 20% AB serum was added and incubated for 4h at 37^{0}C / 5% CO_2. The HUVECS were then washed three times with HBSS and 100µl of neutrophils at 5 x 10^6/ml added and incubated for 30min at 37^{0}C. Non-adherent cells were washed with HBSS and adherent cells were stained with Rose Bengal and the absorbance read at 570nm. The stimulation index in controls (in the absence of emu oil extract) was 4. Statistical analysis by paired t-test, * $P < 0.05$ for comparison between Control and emu oil ethanol extract.*

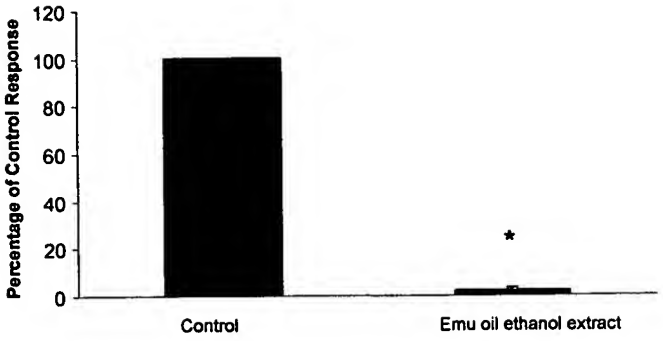


*Fig 23. The effect of Makin emu oil ethanol extract on neutrophil chemotaxis. 2 x 10^6 neutrophils were treated with an equal volume of 1% (v/v) Makin emu oil ethanol extract for 30min at 37^{0}C / 5% CO_2. The cells were centrifuged and resuspended at a density of of 4 x10^7/ml. An agarose plate was set up with the following array in punched holes:5µl DMSO (0.1% v/v) was added to the inner wells, 5µl fMLP (4 x 10^{-7} M) was added to the outer wells and 5µl treated or control neutrophils added to the centre wells and the plate incubated at 37^{0}C / 5% CO_2 for 90 min. Migration of the neutrophils towards fMLP containing wells was measured by inverted microscope. The stimulation index in controls (in the absence of emu oil extract) was 4.4. Statistical analysis by student t-test, * P= 0.004 for comparison between Control and Makin emu oil ethanol extract.*

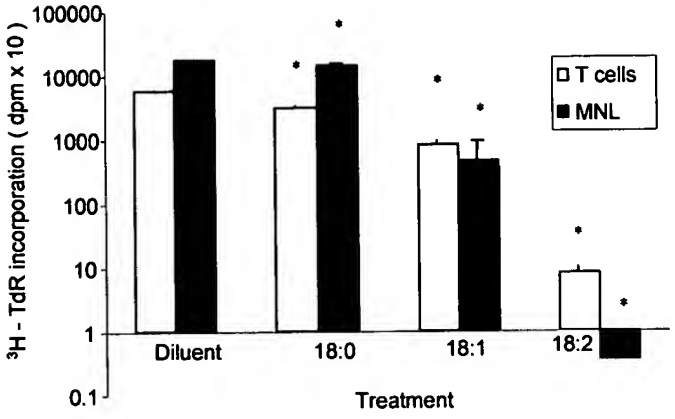


*Fig 24. Effects of 18:0, 18:1ω9 or 18:2ω6 on lymphocyte proliferation. Purified T cells or peripheral blood mononuclear cells (MNL) were preincubated with 20μM of stearic acid (18:0), oleic acid (18:1ω9) or linoleic acid (18:2ω6) for 30 min before being stimulated with PHA/PMA for 72 h. 6 h before the end of the incubation period, cells were pulsed with ^{3}H-thymidine. Following harvesting, the radioactivity incorporated into DNA was determined by liquid scintillation counting. Results are the means ± sem of 3 experiments. A significant inhibition of T cell proliferation was seen only with 18:2ω6. Statistical analysis by Tukey-Kramer multiple comparison test, * P < 0.001 for comparison between fatty acid and Control for purified T cells and MNL. The proliferation stimulation index in controls (absence of fatty acids) was 207.*

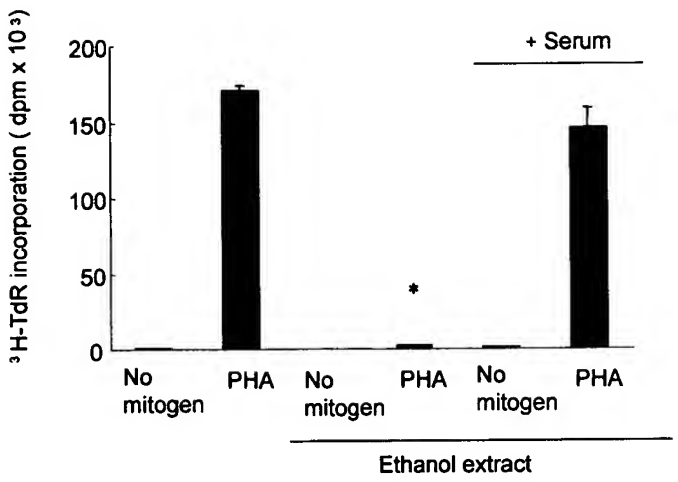


Fig 25. The effect of serum on the Makin ethanol fraction-induced inhibition of lymphoproliferation. To 50µl of purified T lymphocytes (4 x 10^6/ml) was added an equal volume of ethanol or 1%(v/v) ethanol extract of Makin emu oil made up either in RPMI 1640 containing 5% AB serum or in the absence of serum. The cells were incubated at 37^{0}C/5% CO_2/humid atmosphere for 30 min, before 100 µl of 5% AB serum or 2µg/µl PHA (in 5% AB serum) was added to the wells. The wells were then incubated at 37^{0}C/5% CO_2/humid atmosphere for 48 h. Six hours prior to harvesting, cells were pulsed with 1µCi of methyl-^{3}H-thymidine. Incorporated radioactivity was measured using a β counter. The proliferation stimulation index of controls (absence of emu oil extract) was 242. Statistical analysis by Tukey-Kramer Multiple Comparisons Test, $P < 0.001$ for comparison between PHA Control and stimulated Makin ethanol extract minus AB serum.*

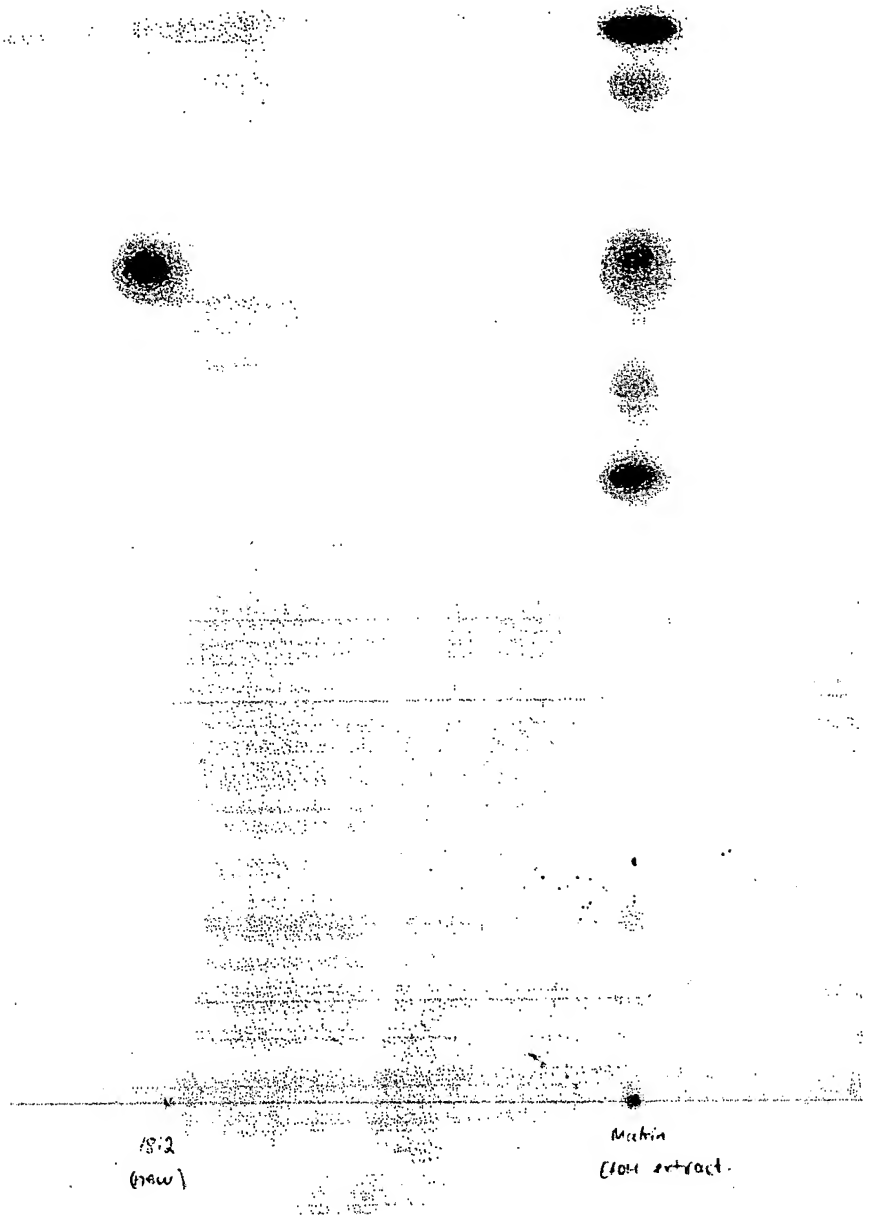


Fig 26. The separation of Makin ethanol extract by TLC. 100μg of 18:2 or Makin ethanol extract was spotted on to a TLC plate and dried under a stream of N_2. The plate was then run in a solvent system (diethyl ether:hexane:glacial acetic acid, 60:40:1, v/v). The plate was allowed to dry and then exposed to I_2 vapour for identification of the zones.

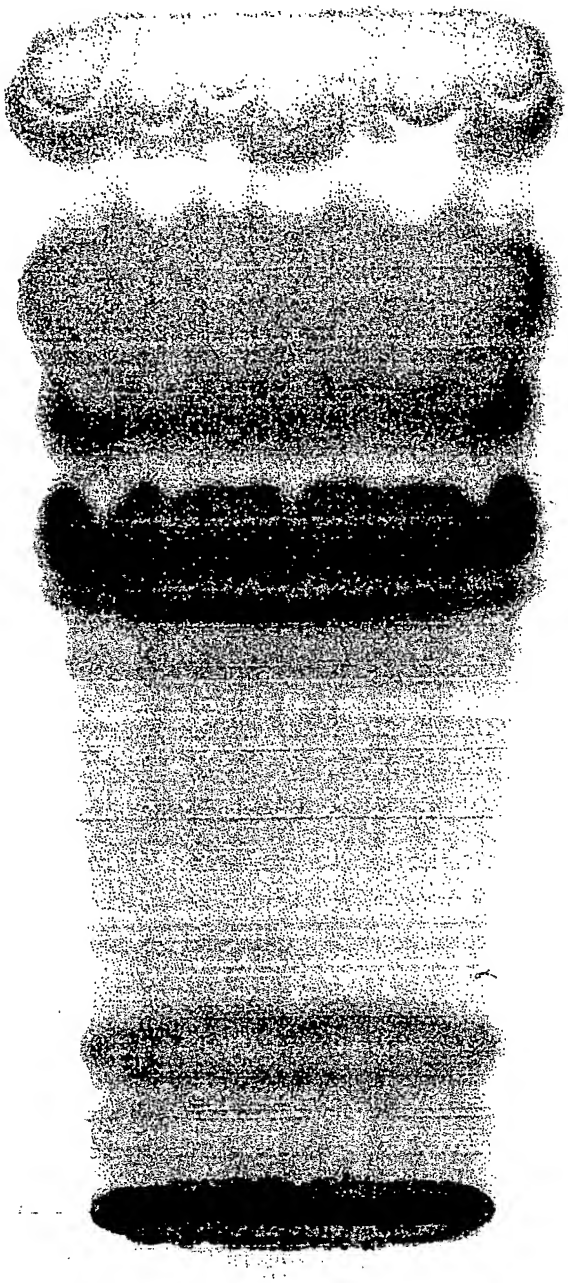


Fig 27. The preparative separation of Makin ethanol extract by TLC. Total ethanol extract of Makin emu oil was spotted onto a TLC plate and dried under a stream of N_2. The plate was then run in a solvent system (diethyl ether:hexane:glacial acetic acid, 60:40:1, v/v). The plate was allowed to dry and then exposed to I_2 vapour for identification of the zones.

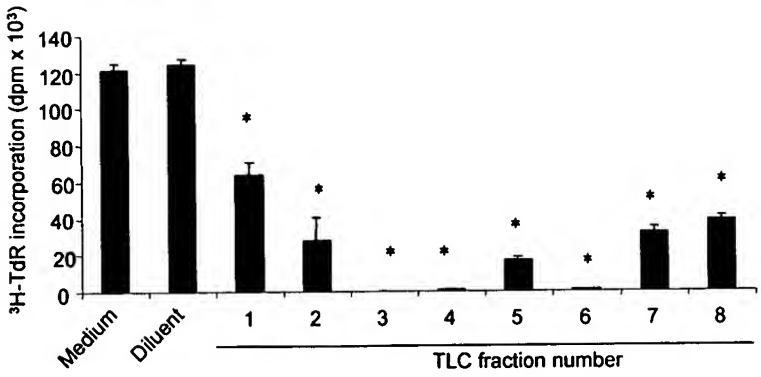


*Fig 28. The effect of TLC separated fractions of Makin ethanol extract on lymphoproliferation. Isolated lipid fractions were dissolved at a concentration of 20mg/ml in ethanol. To 100ml of 4x10^6/ml lymphocytes was added 50ml of ethanol emu oil extract to a final concentration of 1% equivalent of whole oil. Cells were incubated for 30 min in 37°C/5% CO_2 and then stimulated with 2μg/ml PHA for 48 h. 6 h prior to harvesting, cells were pulsed with 1μCi of ^{3}H-thymidine. Cells were harvested and incorporated radioactivity measured by liquid scintillation counting. The proliferation stimulation index in controls (absence of emu oil extract) was 133. Statistical analysis by Tukey - Kramer Multiple Comparisons Test, * $P < 0.001$ for comparisons between Control and TLC fractions.*

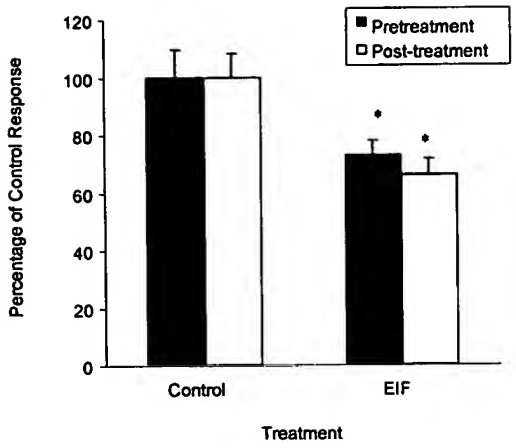


*Fig 29. The effect of ethanol insoluble emu oil fraction (EIF) pre-treatment and post treatment on the DTH response. Mice were injected ip with 120μl of EIF either 4h before (pre-treatment), or 3h after (post treatment), challenge. Mice were challenged in the hind paw with SRBC 5 days after priming. Control mice received PBS. The swelling of the paw was measured after 24h by dial calliper. The results are the Mean ± sem of 5 mice. The Mean ± sem of controls was 1.0 ± 0.08 mm. Statistical analysis by Dunnett's test for Multiple Comparisons, * $P < 0.05$ for comparisons between pre-treatment Control/ EIF and post-treatment Control / EIF.*